COMPARATIVE STUDY ON AMINO ACIDS, FATTY ACIDS, FUNCTIONAL PROPERTIES AND BLOOD CHOLESTEROL STATUS OF RATS FED ON RAW, GERMINATED AND FERMENTED WHITE MELON SEED (*CUCUMEROPSIS MANNII* NAUDIN) FLOUR

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Abstract
White melon seeds were processed as raw (RMF), germinated (GMF) and fermented (FMF) flours. The nutritional qualities and blood cholesterol status of rats fed on flour samples at 10, 20 and 40% concentration were determined. Data were analysed using statistical package, and means were separated using Duncan New Multiple Range test ($p<0.05$). Protein (g/100g) and energy value (kcal.) of FMF (46.26; 577.78) were significantly ($p<0.05$) higher than RMF (39.25; 535.88) and GMF (33.63; 503.15). Mineral in FMF was higher in Na, Cu, Zn, Fe and Ca, but low in K and P than in RMF and GMF sample. Total essential amino acid (g/100 g protein) in FMF (39.8) was significantly ($p<0.05$) higher than GMF (31.29) and RMF (36.2), respectively. Essential amino acid index and predicted biological value of FMF (74.15%; 69.12%) were higher than RMF (69.5%; 64.0%) and GMF (60.7%; 54.5%). The polyunsaturated/saturated fatty acid ratio of GMF (1.77) was higher than RMF (1.71) and FMF (1.73). Antinutrients in FMF were lower than in RMF and GMF, except for phytate. The rats fed on GMF had the lowest blood cholesterol concentration (mg/dL) than rats fed on RMF and FMF. This study established that fermented white melon seed flour had the best nutritional qualities, while germinated flour was rated next in terms of biological value and low antinutrients. However, rats fed on fermented white melon seed flour had the highest blood cholesterol concentration increased than raw and germinated flour, respectively. Hence, germinated white melon seed flour is nutritional suitable for consumption.

Keywords: Germination and fermentation techniques, White melon kernels, Nutritional quality, Blood cholesterol

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INTRODUCTION

White melon (*Cucumeropsis mannii*) is a cucurbit crop with fibrous and shallow root system that belongs to the cucurbitaceae family. *Cucumeropsis mannii* is propagated entirely by seed in the tropical savannah zone of West Africa during the major rainy season between March and May (Egunjobi and Adebisi, 2004). Propagation starts after the first couple heavy rainfalls of the season, and after 6–8 months, around September–December the fruit are ready for harvesting (Egunjobi and Adebisi, 2004).

Melon is a major food crop with several varieties, which serve as a major source of protein and oil (Mabalaha et al., 2007). A valuable vegetable oil is extracted from the seeds, while the ground seed is used to prepare various delicacies including cake and soup (Onyeike and Achera, 2001). The kernel of white melon seed contains essential nutrients like oil, protein, carbohydrate and appreciable amount of micronutrient (Mabalaha et al., 2007). The oil of white melon seed is high in fatty acids like linoleic acid, oleic acid, stearic acid and palmitic acid (Idowu *et al.*, 2003). White melon seed is the perfect complement to the largely starch-rich grain diet of Africa, providing a high- protein and high-energy concentrate (Idowu *et al.* 2003). The amino acid content of melon seed protein makes it a sufficient vegetable protein for the growing children and adults (Kalac et al., 1991). There is potential for these seeds as a critical tool for intervention in protein-energy malnutrition, and that intake of the seed (100g) will provides essential fatty acid, amino acid and vitamin E of daily requirements (Kalac et al., 1991).

Despite nutritional composition of white melon seed, the seed is underutilised and there is dearth information on the chemical composition, antioxidants and influence of its consumption on blood lipid profile. Hence, the present study aimed at evaluating nutritional composition, functional properties and influence of white melon seed on blood cholesterol profile.

MATERIALS AND METHODS

Source of Food Materials

The white melon seeds (*Cucumeropsis mannii* Naudin) were purchased from Erekesan market, Akure, Ondo State, Nigeria, and identified in the Department of Crop, Soil and Pest, Federal University Technology, Akure, Nigeria.

Processing of Food Materials into Flour

Raw white melon seed flour processing

Raw white melon seed was processed into flour using modified method of Peter-Ikechukwu et al. (2016). White melon seeds were sorted to remove unwanted materials like stones, pebbles and other foreign seeds, dehulled, washed with double distilled water and drained. The kernels were oven dried at 60 ^{o}C for 20 h using a hot-air oven (Plus11 Sanyo Gallenkamp PLC, Loughborough, Leicestershire, UK), milled with a laboratory blender (Model KM 901D; Kenwood Electronic, Hertfordshire, UK) and passed through a 60 mm mesh sieve (British Standard) to obtain raw white melon seed flour. The flour was packed in a plastic container, sealed and stored at room temperature (~27 ^{o}C) until analysis.

Fermented white melon seed flour processing

Raw white melon seed was processed into fermented flour using modified method of Ejinkeonye et al. (2018). White melon seeds were sorted to remove unwanted materials like stones, pebbles and other foreign seeds, dehulled, washed with double distilled water and drained. The kernels were boiled in distilled water (1: 2) with a pot, for 6 h to aid softening. Intermittently, water was added to the pot to prevent burning. Then, on completion of boiling, the seeds were drained and allowed to cool for 30 min. After cooling, the seeds were mashed and wrapped in plantain leaves (Musa spp). The plantain leaves before usage, were flamed to make them pliable in order to prevent breakage. After that, samples were put into a clean sack bag and incubated at ambient temperature for 72 h. The fermented melon was oven dried at 60 ^{o}C for 20 h using a hot-air oven (Plus11 Sanyo Gallenkamp PLC, Loughborough, Leicestershire, UK), milled with a laboratory blender (Model KM 901D; Kenwood Electronic, Hertfordshire, UK) and passed through a 60 mm mesh sieve (British Standard) to obtain fermented white melon seed flour. The flour was packed in a plastic container, sealed and stored at room temperature (~27 ^{o}C) until analysis.

Germinated white melon seed flour processing

Germinated white melon seed flour was processed into germinated flour using modified method of Adelekan and Oyewole (2010). White melon seeds were sorted to remove unwanted materials like stones, pebbles and other foreign seeds, soaked in distilled water for 6 h and drained. The kernels were spread on moisten muslin bag and cover with another muslin bag, watered and left to germinate in a dark cupboard at room temperature (30 ± 1°C) for 3- 4 days. After germination, the seeds were dehulled, re-washed, oven dried at 60 ^{o}C for 20 h using a hot-air oven (Plus11 Sanyo Gallenkamp PLC, Loughborough, Leicestershire, UK), milled with a laboratory blender (Model KM 901D; Kenwood Electronic, Hertfordshire, UK) and passed through a 60 mm mesh sieve (British Standard) to obtain germinated white melon seed flour. The flour was packed in a plastic container, sealed and stored at room temperature (~27 ^{o}C) until analysis.

Determination of proximate composition of white melon seed flour

Proximate compositions, that is, moisture content, ash, crude fiber, crude fat and crude protein content of experimental food samples were determined using the standard methods (AOAC, 2012). Carbohydrate content was determined by difference as follow:

Carbohydrate (%) = 100-(%Moisture + %Fat + %Ash + % Crude fibre + %Crude protein)

The gross energy values of the samples were determined (MJ/kg) by using Gallenkamp Adiabatic bomb calorimeter (Model CBB-330-01041; UK).

Determination of anti-nutritional factor of white melon seed flour

Flavonoid, tannin, phenols, saponin. phytate and oxalate were determined out using methods of AOAC (1990). Trypsin inhibition activity was determined as described by Griffiths (2000).

Determination of amino acid composition of processed white melon seed flour

The amino acid profiles of the experimental samples were determined according to the method described by AOAC (2012). The experimental samples were digested using 6N HCl for 24 h. Amino acids were determined using the Beckman Amino Acid Analyzer (model 6300; Beckman Coulter Inc., Fullerton, Calif., USA) employing sodium citrate buffers as step gradients with the cation exchange post-column ninhydrin derivatization method. The data were calculated as grams of amino acid per 100 g crude protein of flour sample.

Determination of fatty acids composition of white melon seed flour

The composite flour samples were extracted with chloroform: methanol (2:1v/v) and solid non-liquid material was removed by filtration. The total extracted lipid material was recovered after solvent removal in a stream of nitrogen. The samples were re-dissolved in anhydrous chloroform/methanol (19:1 v/v), and clarified by centrifugation at 10,000 x *g* for 10 min. Tranmethylation was performed using 14% (w/v) boron triflouride (BF_3) in methanol (Solomon and Owolawashe, 2006). Fifty nanograms of heptadecanoic acid (internal standard) and 1mL aliquot of each sample were transferred to a 15 mL Teflon-lined screw-cap tube. After removal of solvent by nitrogen gassing, the samples were mixed with 0.5 ml of BF_3 reagent (14% w/v), placed in warm bath at 100 °C for 30 min and cooled. After the addition of saline solution, the transmethylated fatty acids were extracted into hexane. A calibration mixture of fatty acid standards was processed in parallel. Aliquots of the hexane phase were analyzed by gas chromatography. Fatty acids were separated and quantified using a Hewlett-Packard gas chromatograph (5890 Series II) equipped with a flame-ionization detector. Two microliter aliquot of the hexane phase were injected in split-mode onto a fused silica capillary column (Omegawax: 30 m x 0.32 mm ID, Supleco, Bellefonte, PA). The injector temperature was set at 200 °C, detector at 230 °C, oven at 120 °C initially, then 120-205 °C for 18min.The carrier gas was helium and the flow rate was approximately 50 cm/sec. Electronic pressure control in the constant flow mode was used. The internal standard (heptadecanoic acid, C17:0) and calibration standards (NuCheck, Elysian, MN) were used for quantization of fatty acids in the lipid extracts. The fatty acids reported represent the average of three determinations.

Determination of Functional Properties of Melon Flour Blends

Determination of bulk density

The bulk density was determined using the modified method of Narayana and Narasinga Roa (1984). A known quantity of the flour mixes was put into a known weighed 5 ml measuring cylinder (W_1). The measuring cylinder was gently tapped to eliminate air spaces between the flour mixes in the measuring cylinder and the volume was noted to be the volume of the sample used. The new mass of the sample and the measuring cylinder were recorded as (W_2). The Bulk density (BD) was expressed as:

$$\text{BD} = \frac{W2 - W1}{Volume\ of\ sample\ used}\, x\ 100$$

Determination of water absorption capacity (WAC)

The water absorption capacity (WAC) was determined according to the method of Tomotake *et al.*, (2002) with minor modifications. Each protein sample (1 g) was weighed into 25 mL pre-weighed centrifuge tubes. Distilled water (10 mL) was added to

each sample in small increments to a series of tubes that were under continuous stirring using a glass rod. After the mixture was thoroughly mixed, the samples were centrifuged at 9,500 ×g for 30 min. After centrifugation the supernatant was decanted and the weight recorded.

WHC (which is grams of water per gram of protein) was calculated as:

$$\text{WAC} = \frac{(W_2 - W_1)}{W_0}$$

Where:

W_0 = weight of the dry sample (g)

W_1 = weight of the test tube and dry sample (g)

W_2= weight of the test tube and the paste (g)

Determination of Oil absorption capacity (OAC)

Oil absorption capacity (OAC) was determined using the method of AOAC (2012). The sample (W_0) (1.0 g) was weighed into pre-weighed 15 ml centrifuge tubes and thoroughly mixed with 10 mL (V_1) of refined pure groundnut oil using vortex mixer. Samples were allowed to stand for 30 min. The sample-oil mixture was centrifuged at 3,000 *xg* for 20 min., and immediately after centrifugation, the supernatant was carefully poured into a 10 mL graduated cylinder, and the volume was recorded (V_2). OAC (milliliter of oil per gram of sample) was calculated using Equation below:

$$\text{OAC} = \frac{(V_2 - V_1)}{\text{W}_0}$$

Where:

W_0 = weight of the dry sample (g)

V_1 =weight of the tube plus the dry sample (g)

V_2 = weight of the tube plus the sediment (g)

Determination of swelling capacity (SC)

The swelling capacity was determined by the method modified by Abraham (1993). Samples (5g) were weighed into dry test tube 50ml centrifuge tube. Double distilled water (30 mL) was added and mixed gently. The slurry was heated in water bath at desire temperatures- 40, 50, 60, 70, 80and 90 ^{0}C for 30 min. in a thermostat water bath. During heating, the slurry was stirred gently to prevent clumping of the starch. After 30 min, the tube containing the paste was cooled and centrifuged at 9,500 $\times g$ for 30 min. The supernatant was decanted immediately after centrifugation. The tubes were dried at 50 ^{o}C for 30 min, cooled and then weighed (W_2). Centrifuge tubes containing sample alone were weighed (W_2). Centrifuge tubes containing sample alone were weighed prior to adding distilled water (W_1). Swelling capacity was calculated as follows:

$$\text{SC} = \frac{W2 - W1}{Weight\ of\ sample} x\ 100$$

Determination of foaming capacity (FC)

The foaming capacity (FC) was determined as described by Peter-Ikechukwu et al. (2016) with slight modification. The white melon seed flour (1.0 g) sample was added to 50 mL distilled water at 30±2 °C in a graduated cylinder. The suspension was mixed and shaken for 5 min to foam. The volume of foam at 30 s after whipping was expressed as foaming capacity using the formula:

$$\text{FC} = \frac{Vol\ after\ whipping - vol\ before\ whipping}{Volume\ before\ whipping} x\ 100$$

Determination of emulsion capacity (EC)

The emulsion activity was determined as described by Peter-Ikechukwu et al. (2016). The white melon flour (1 g) was mixed with 10 mL distilled water and 10 mL soybean oil in calibrated centrifuge tube. The emulsion was centrifuged at 2000 × g for 5 min. The volume of oil separated from the sample after centrifugation was read directly from the tube. The ratio of the height of emulsion layer to the total height of the mixture was calculated as emulsion capacity in percentage.

Determination of blood cholesterol level

Animal treatment

Wistar albino rats, male and female, weighing between 50-55 g were grouped into 3 groups consisting of 7 rats each, and the rats were allowed to acclimatize with the new environment for 7 days with free water and commercial animal feed. The rats were fed with cholesterol-induced diets, basal diet and

water *ad libitum* for 28 days. At the end of the experimental period (28 days), the animals were fasted overnight and sacrificed under chloroform anaesthesia. Blood was collected from each rat via cardiac punctured and transferred into lithium heparin bottle. The collected blood samples were immediately spun at 3000 *x g* to collect the plasma portion which was used to determine blood lipid profile of the animals.

Determination of blood cholesterol level

The blood samples were centrifuged at 3000 xg for 15 min at room temperature. Separated top layer of serum was used for the analysis. Total cholesterol was measured using established enzymatic methods of Allain *et al*. (1974) with the Randox cholesterol kit (Randox England). The serum (10 µL) sample was prepared by mixing with assay reagent (1.0 mL). Standard sample was prepared by mixing a 200 mg/dl cholesterol solution (10 µL) with assay reagent (1.0 mL). Blank sample was prepared by mixing distilled water (20 µL) with assay reagent (1.0 mL). The mixtures were incubated at 37 ^{o}C for 5 min. and the absorbance was read at 500 nm using an Ultra Violet visible spectrophotometer (Optima SP-3000 plus, Tokyo, Japan). The cholesterol content was calculated using the following equation.

$$\text{Cholesterol} = \frac{\text{C x 20 x DF x W}}{\text{4 x 100}}$$

Where:
C=concentration of cholesterol (from standard curve);
DF = dilution factor;
W = weight of sample

Statement of Animal Rights

The study protocol was approved by the Ethical Committee for Laboratory Animals of School of Agriculture and Agricultural Technology, Akure, Nigeria (FUTA/SAAT/2018/011). The experiments on animals were conducted in accordance with the force laws and regulations as regards animal use and care as contained in the Canadian Council on Animal Care Guidelines and Protocol Review (CCAC, 1993).

RESULTS AND DISCUSSION

The proximate and mineral composition of raw, germinated and fermented white melon seed flour samples are presented in Table 1. The moisture content of flour samples ranged from 2.16 g/100g in fermented white melon seed flour (FMF) to 3.12 g/100g in raw melon seed flour (RMF). The moisture content in white melon seed flour samples was comparatively lower than FAO/WHO (1991) recommended value (<10 mg/100g) for flour samples. This observation showed that low moisture content of white melon seed flour may inhibits the growth of microorganisms and thereby prolong the shelf life of the flour products. The protein and fat content of fermented white melon seed flour (FMF) (46.26, 35.95 g/100g) were significantly ($p<0.05$) higher than raw seed flour (RMF) (39.25, 31.92 g/100g) and germinated seed flour (GMF) (33.63, 28.08 g/100g). The high protein content of fermented white melon seed flour could be attributed to the activities of microorganisms. It is evident that during fermentation process microorganisms usually synthesized protein from other nutrients like carbohydrate for its growth and biochemical activities and multiplications (Iheke et al., 2017). Similarly, the fat content of fermented white melon seed flour was higher than RMF and GMF, and this may be due to increase in activities of lipolytic enzymes, which hydrolyze fat to glycerol and fatty acid (Iheke et al., 2017; Bello and Udo, 2017). Comparatively, the protein and fat content of white melon seed flour samples in this study were within the range values of what reported by Peter-Ikechukwu et al. (2016) and Peter (2018). The energy value of FMR (577.78 kcal.) was significantly higher than RMF (535.88 kcal.) and GMF (503.15 kcal.), respectively. This finding could be attributed to the high value of fat and protein in the fermented flour sample when compared with the RMF and GMF samples. Nutritionally, application of white melon seed flour in food formulation, particularly infant food, would improve the protein and energy value of the products, and thereby prevent protein-energy malnutrition among young children.

Table 1: Proximate (g/100g dry wt.) and minerals (mg/100g) composition of raw, germinated and fermented white melon seed flour

Parameters	RMF	GMF	FMF
Moisture	3.12±0.02^{a}	2.57±0.01^{b}	2.16±0.02^{c}
Fat	31.92±0.46^{b}	28.08±2.12^{b}	35.95±0.91^{a}
Protein	39.25±1.30b	33.63±2.82^{b}	46.26±1.41^{a}
Ash	1.96±0.01b	3.36±0.27^{a}	2.37±0.20^{b}
Fiber	13.07±0.04^{a}	11.35±1.62^{a}	10.99±0.44^{a}
CHO	13.75±1.69^{a}	22.04±4.74^{a}	4.41±0.15^{b}
Energy	535.88±6.48^{ab}	503.15±10.13^{b}	577.78±9.54^{a}
Na	1.16±0.01^{b}	0.92±0.01^{c}	1.23±0.01^{a}
Cu	0.31±0.01^{c}	0.71±0.01^{b}	0.91±0.01^{a}
Zn	10.81±0.10^{b}	12.28±0.18^{a}	12.83±0.12^{a}
Fe	6.77±0.06^{c}	7.62±0.11^{b}	8.89±0.09^{a}
Ca	2897.69±28.69^{b}	4317.81±63.81^{a}	4336.94±42.94^{a}
K	558.025±5.53^{a}	499.89±7.39^{b}	361.08±3.57^{c}
P	290.78±2.88^{b}	317.19±4.69^{a}	306.23±3.03^{ab}
Na/K	0.00±0.00	0.000.00	0.00±0.00
Ca/P	1.00±0.00^{c}	1.36±0.00^{b}	1.42±0.00^{a}

Means (±SEM) with different superscripts (a-c) in the same row show significant difference at $p < 0.05$. RMF (Raw white melon seed flour), GMF (Germinated white melon seed flour), FMF (Fermented white melon seed flour).

The mineral composition of fermented white melon seed flour (FMF) was higher in Na, Cu, Zn, Fe and Ca, but low in K and P when compared to RMF and GMF sample. The most abundant mineral in white melon seed flour was calcium, and the value ranged from 2897.69 mg/100g in RMF to 4336.94 mg/100g in FMF, while the least element was copper ranged from 0.31 mg/100g in RMF to 0.91 mg/100g in FMF. Nutritionally, white melon seed flour contains essential minerals like Zn, Fe, Ca and P, which are important for cognitive, blood and bone formation and development, particularly in children (Soetan et al., 2010).

The amino acid composition and nutritional quality of the raw, germinated and fermented melon seed flour samples are presented in Table 2. The total essential amino acid composition of fermented melon seed flour was 39.8 g/100 g protein, and the value was significantly higher than RMF (36.2 g/100g protein), GMF (31.29 g/100g) and that of FAO/ WHO (1991) recommended value (30.1 g/day). For the total non-essential amino acid composition, RMF (58.87 g/100g) had the highest concentration when compared with FMF (57.73 g/100g) and GMF (49.99 g/100g). In this study, glutamic acid was observed to be the most abundant amino acid, while lysine was the first limiting essential amino acid in white melon seed flour. The high value of glutamic acid in white melon seed flour agreed with the reports that this amino acid is usually the most predominant in plant-based foods (Ogunmodimu *et al*., 2015).

Recently, scientific study has shown that intakes of vegetable proteins that high in amino acids like glutamic acid, arginine, glycine, cysteine, histidine, and tyrosine have the

Table 2: Amino Acid profiles (g/100 g of protein) of raw, germinated and fermented white melon seed flour

Parameters	RMF	FMF	GMF	*REF.
Essential amino acids (EAA)				
Valine	4.82±0.03^{b}	5.23±0.03^{a}	4.23±0.02^{c}	3.5
Threonine	3.02±0.02^{a}	3.02±0.02^{a}	2.28±0.01^{b}	3.4
Isoleucine	5.33±0.03^{a}	5.07±0.03^{b}	4.87±0.03^{c}	2.8
Leucine	7.63±0.04^{b}	9.33±0.05^{a}	6.51±0.03^{c}	6.6
Lysine	3.47±0.02^{b}	4.59±0.03^{a}	2.94±0.02^{c}	5.8
Methionine	2.85±0.02^{a}	2.53±0.02^{b}	2.41±0.01^{c}	2.2
Phenylalanine	4.99±0.03^{b}	5.71±0.03^{a}	4.45±0.02^{c}	2.8
Histidine	2.50±0.01^{b}	2.58±0.02^{a}	2.12±0.01^{c}	1.9
Tryptophan	1.59±0.01^{b}	1.74±0.01^{a}	1.48±0.01^{c}	1.1
ΣEAA	36.2±0.40^{b}	39.8±0.44^{a}	31.29±0.34^{c}	30.1
Non-essential amino acids (NEAA)				
Arginine	14.70±0.08^{b}	15.74±0.08^{a}	13.32±0.07^{c}	2.0
Alanine	4.88±0.03^{a}	4.34±0.02^{b}	3.89±0.02^{c}	-
Serine	3.64±0.02^{b}	4.02±0.02^{a}	3.10±0.02^{c}	-
Proline	3.88±0.02^{b}	4.28±0.02^{a}	3.06±0.02^{c}	-
Glutamate	15.07±0.08^{a}	14.00±0.07^{b}	12.64±0.07^{c}	-
Glycine	4.64±0.03^{a}	3.92±0.02^{c}	4.25±0.02^{b}	-
Tyrosine	3.29±0.02^{a}	1.90±0.01^{c}	2.07±0.01^{b}	-
Aspartate	8.29±0.04^{b}	8.92±0.05^{a}	7.30±0.04^{c}	-
Cysteine	0.48±0.00^{b}	0.61±0.01^{a}	0.36±0.00^{c}	-
ΣNEAA	58.87±0.65^{a}	57.73±0.64^{b}	49.99±0.55^{c}	-
Predicted Nutritional Qualities				
PER	3.06^{b}	3.36^{a}	2.67^{c}	2.5
EAAI (%)	69.5^{b}	74.15^{a}	60.7^{c}	70
Predicted BV (%)	64.0^{b}	69.12^{a}	54.5^{c}	70
Nutritional index (%)	27.3^{b}	34.3^{a}	20.4^{c}	-
ΣSAA(Meth+Cys)	3.33^{a}	3.14^{b}	2.77^{c}	-
ΣArAA(Phe+Tyr)	8.28^{a}	7.61^{b}	6.52^{c}	-
Lysine/ Arginine	0.24^{b}	0.29^{a}	0.22^{c}	-
Essential Amino Acid Scores				
1st LAA	Lysine	Lysine	Lysine	-
AAA	Arginine	Arginine	Arginine	-

*Means (±SEM) with different superscripts (a-c) in the same row show significant difference at p < 0.05. RMF (Raw white melon seed flour), GMF (Germinated white melon seed flour), FMF (Fermented white melon seed flour). *FAO/ WHO (1991). 1st Limiting Amino Acid (LAA), Abundant Amino Acid (AAA), protein efficient ratio (PER), Essential amino acids index (EAAI), Biological value (BV), Sulphur amino acids (SAA), Aromatic amino acids (ArAA)*

potentials of lowering both systolic and diastolic blood pressure (Tuttle et al., 2012).The protein efficiency ratio (PER), essential amino acid index (EAAI) and predicted biological value (BV) of fermented white melon seed flour (3.36; 74.15%; 69.12%) were higher than the values obtained for RMF (3.06; 69.5%; 64.0%) and GMF (2.67; 60.7%; 54.5%), respectively. Nutritionally, the BV and EAAI of fermented white melon seed flour were comparable to that of FAO/ WHO (1991) recommended values, that is, 70%. This implies that application of fermentation in processing white melon seed flour would thereby improve its nutritional quality of the product, this finding agreed with the report of Bello and Udo (2017) who reported that fermentation improved the nutritional properties, reducing anti- nutrients content and also enhancing the functionalities of horse eye beans flour.

The fatty acid composition of raw, germinated and fermented white melon seed flour is presented in Table 3. The range values of total saturated, monounsaturated and polyunsaturated fatty acid composition were 20.79 - 28.56%, 9.79 - 14.26% and 35.58 - 50.46%, respectively.

Table 3: Fatty acid composition (%) of raw, germinated and fermented white melon seed flour

Parameters	RMF	FMF	GMF
Saturated FA			
Behenic	0.01±0.01^{c}	0.07±0.01^{b}	0.13±0.01^{a}
Caproic	0.00±0.00^{b}	0.04±0.02^{ab}	0.07±0.01^{a}
Capylic	0.00±0.00^{a}	0.05±0.02^{a}	0.07±0.02^{a}
Capric	0.00±0.00^{b}	0.02±0.01^{ab}	0.06±0.02^{a}
Lauric	1.14±0.01^{c}	1.23±0.02^{b}	1.62±0.01^{a}
Lignoceric	0.00±0.00^{b}	0.03±0.01^{b}	0.07±0.01^{a}
Margaric	0.02±0.01^{b}	0.06±0.01^{ab}	0.10±0.01^{a}
Mystric	0.61±0.02^{c}	1.00±0.03^{b}	1.13±0.02^{a}
Palmitic	10.07±0.02^{c}	13.24±0.01^{b}	13.51±0.03^{a}
Stearic	8.95±0.02^{c}	10.36±0.01^{b}	11.81±0.02^{a}
∑SFA	20.79±3.02^{c}	26.09±2.01^{b}	28.56±3.21^{a}
Monounsaturated FA			
Erucic	0.04±0.01^{b}	0.11±0.02^{b}	0.19±0.02^{a}
Oleic	9.75±0.02^{c}	12.80±0.02^{b}	14.01±0.04^{a}
Palmitoleic	0.00±0.00^{b}	0.02±0.01^{b}	0.06±0.01^{a}
∑MUFA	9.79±1.13^{c}	12.93±2.00^{b}	14.26±1.02^{a}
Polyunsaturated FA			
Linoleic	34.54±0.02^{c}	43.81±0.03^{b}	48.71±0.02^{c}
Arachidonic	0.82±0.24^{a}	1.04±0.01^{a}	1.20±0.02^{a}
Linolenic	0.22±0.01^{c}	0.34±0.02^{b}	0.56±0.02^{a}
∑PUFA	35.58±3.02^{c}	45.19±2.06^{b}	50.46±3.21^{a}
MUFA/PUFA	0.28±0.00^{b}	0.29±0.00^{a}	0.28±0.00^{b}
PUFA/SFA	1.71±0.01^{c}	1.73±0.02^{b}	1.77±0.00^{a}
(MUFA+PUFA)/SFA	2.18±0.04^{c}	2.23±0.0^{b}	2.27±0.03^{a}

Means (±SEM) with different superscripts (a-c) in the same row show significant difference at p < 0.05. RMF (Raw white melon seed flour), GMF (Germinated white melon seed flour), FMF (Fermented white melon seed flour).

These parameters were significantly ($p<0.05$) higher in germinated flour sample than raw and fermented flour sample. This finding agreed with the report of Huang and Grunwald (1990), who reported that germination technique increased fatty acid composition of alfalfa seeds. The polyunsaturated/saturated fatty acid (P/S) ratio of germinated white melon seed flour was 1.77, and the value was significantly higher when compared with raw (1.71) and fermented flour sample (1.73). This observation implies that white melon seed oil contains more of polyunsaturated fatty acid than saturated fatty acids, and this would promote good health to its consumers. Nutritional studies have established that intake of dietary fat high in P/S ratio (>1.0) reduces serum cholesterol and atherosclerosis, hence, lowering both systolic and diastolic blood pressure (Ramadan et al., 2006). The fatty acid composition and high P/S ratio make the white melon seed oils an ideal component for nutritional applications. The antinutrient composition of raw, germinated and fermented white melon seed flour is shown in Table 4. The antinutrient composition (mg/100g), that is, oxalate, phytate, tannin, phenol, saponin and flavonoid concentration in white melon seed flour ranged as follows: 0.31 - 0.99, 24.31 - 26.78, 1.93 - 2.49, 7.69 - 16.11, 5.73 - 18.28 and 1.59 - 3.98, respectively. The present study showed that antinutrient concentration in FMF was significantly ($p<0.05$) lower, except in phytate, when compared with RMF and GMF flour samples. The reduction in antinutrient concentration in fermented white melon seed flour could be attributed to various stage that involved in fermentation process, such as soaking, boiling and microbial activities. This finding agreed with the report of Bello and Udo (2017), who also reported that fermentation technique reduced antinutrients in horse eye bean flour. The reduction of antinutrient level like oxalate in white melon seed flour had great nutritional and health benefits such as enhancing the bio-availability of protein and minerals (Ca, Zn & Fe). Besides, it is well established that intakes of phytochemicals like saponin, flavonoid, phenol, etc. in small quantities are effective in the treatment and prevention of many diseases like coronary heart diseases, obesity, diabetes, and gastrointestinal diseases (Sabiu et al., 2017).

Functionality of foods is the characteristics of food ingredient other than nutritional quality, which has a great influence on its utilization (Mahajan and Dua, 2002). The functional properties of raw, germinated and fermented white melon seed flour is presented in Table 5. The range values of water absorption capacity (WAC), oil absorption capacity (OAC), bulk density (BD), emulsion capacity (EC) and foaming capacity were 139.21 -176.40%, 137.21 - 179.67%, 0.49 - 0.83 g/mL, 43.66 - 64.02% and 8.19 - 27.89%, respectively; while that of dispersibility ratio and swelling capacity were 28.89 – 45.96% and 31.33 – 46.33%, respectively.

Table 4: Antinutrient composition of raw, germinated and fermented white melon seed flour

Parameters	RMF	FMF	GMF
Oxalate**(mg/100g)**	0.99 ± 0.09^{a}	0.41 ± 0.05^{c}	0.72 ± 0.00^{b}
Phytate**(mg/100g)**	26.78 ± 1.24^{a}	24.72 ± 0.00^{a}	24.31 ± 0.41^{a}
Tannin**(mg/100g)**	2.49 ± 0.01^{a}	1.93 ± 0.02^{c}	2.08 ± 0.02^{b}
Phenol**(mg/100g)**	16.11 ± 0.07^{a}	7.69 ± 0.13^{c}	9.45 ± 0.03^{b}
Saponin (%)	18.28 ± 0.27^{a}	5.73 ± 0.09^{c}	13.64 ± 0.19^{b}
Flavonoid**(mg/100g)**	3.98 ± 0.03^{a}	1.59 ± 0.04^{c}	3.10 ± 0.03^{b}

Means (±SEM) with different superscripts (a-c) in the same row show significant difference at $p < 0.05$. RMF (Raw white melon seed flour), GMF (Germinated white melon seed flour), FMF (Fermented white melon seed flour).

Table 5: Functional properties of raw, germinated and fermented white melon seed flour

Parameters	RMF	GMF	FMF
WAC (%)	137.19±0.05^{b}	139.21±0.04^{b}	176.40±0.04^{a}
OAC (%)	158.03±0.04^{b}	179.67±0.33^{a}	137.21±0.03^{c}
Bulk density (g/mL)	0.68±0.02^{b}	0.83±0.01^{a}	0.49±0.02^{c}
Emulsion capacity (%)	62.03±0.02^{b}	64.02±0.01^{a}	43.66±0.06^{c}
Foaming capacity (%)	20.37±0.02^{b}	27.89±0.06^{a}	8.19±0.02^{c}
Dispersibility ratio (%)	28.89±0.06^{c}	45.96±0.03^{a}	30.00±0.05^{b}
Swelling Capacity (%)	31.33±0.88^{c}	46.33±0.88^{a}	34.67±0.33^{b}

Means (±SEM) with different superscripts (a-c) in the same row show significant difference at $p < 0.05$. RMF (Raw white melon seed flour), GMF (Germinated white melon seed flour), FMF (Fermented white melon seed flour).

Statistically, germinated white melon seed flour (GMF) was significantly ($p<0.05$) higher than RMF and FMF in all determined functional properties, except for WAC. This could be as a result of reactivation of dormant enzymes that produce primary and secondary metabolites, and thereby improving the nutritional and functional properties of the seed (Abbas and Mushara, 2008). In agreement, several authors have reported that germination technique increased nutritional and functional properties of seed flours (Imtiaz and Burhan-Uddin, 2012; Ocheme et al., 2015). For instance, studies have shown that high water absorption capacity as observed in germinated white melon seed flour may be used as thickening agent in soup preparation and baked products, similarly, high oil absorption capacity may also be useful in structural interaction in foods especially in flavour retention, improvement of palatability and extension of shelf life particularly in bakery or meat products where fat absorption is desired (Bello and Udo, 2017). The high bulk density of germinated melon seed flour as observed in this study suggests better packaging properties than those with low bulk density (Fagbemi, 1999). In contrast, low bulk density of a flour sample would be an advantage in the formulation of high nutrient-density complementary foods for convalescent and child feeding (Olawuni et al., 2013).

The blood cholesterol concentration (mg/dL) of Albino rats fed on raw (RMF), germinated (GMF) and fermented (FMF) white melon seed flour at different concentration of 10, 20 and 40% for 28 days is presented in Figure 1.

The blood cholesterol concentration of rats fed on different concentration of white melon seed flours during the experimental period ranged from 17.48 to 31.88, 30.33 to 68.38 and 43.67 to 125.96 mg/dL for the group of rats fed on 10% and 40% of GMF, RMF and FMF, respectively. In this study, it was observed that as the concentration of white melon seed flour increased, the blood cholesterol concentration of the rats likewise increased. Besides, the finding in this study also showed that the rats fed on germinated white melon seed flour had the lowest blood cholesterol concentration, while that of fermented seed flour had the highest cholesterol increased, and this observation agreed with the report of Lopes et al. (2018). The low blood cholesterol that was observed in rats fed on germinated melon seed flour could be attributed to the high concentration of polyunsaturated fatty acid, bioactive proteins (peptides) and phytochemicals present in the seed. Several studies have shown that these bioactive compounds regulate the activities of key enzymes, which involved in cholesterol metabolism, such as fatty acid synthetase, 3-hydroxy-3-methyl-glutaryl-CoA reductase

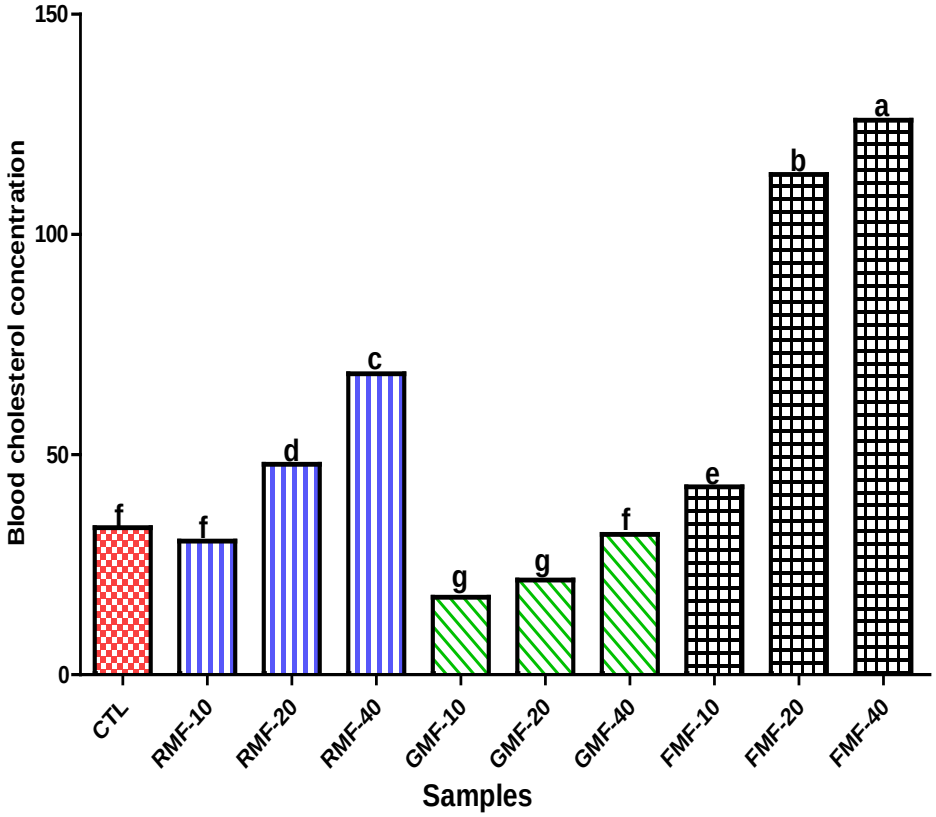

Fig 1: Blood cholesterol concentration (mg/dL) of Albino rats fed on Control (CTL), Raw white melon seed flour ($RMF_{10, 20, 40\%}$), Germinated white melon seed flour ($GMF_{10, 20, 40\%}$), Fermented white melon seed flour ($FMF_{10, 20, 40\%}$) at different contration for 28 days

(HMG-CoA), HMG-CoA synthetase, and cholesterol 7-α-hydroxylase (Yao et al., 2014; Marques et al., 2015b).Epidemiological evidences have shown that most diets in developing countries are characterized with high saturated fatty acids, which contributes significantly to the risk of developing atherosclerotic heart disease and cardiovascular disease in humans (Romero-Corral et al., 2006; Krivanek et al., 2007), hence, consumption of white melon seeds, particularly fermented melon seed products, should be taken with caution.

CONCLUSION

In conclusion, this study established that fermented white melon seed flour had the best nutritional qualities, while germinated flour was rated next in terms of biological value and low antinutrients. However, rats fed on fermented white melon seed flour had the highest blood cholesterol concentration increased than raw and germinated flour, respectively. Hence, germinated white melon seed flour is nutritional suitable for consumption.

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